

Biological attributes of diapausing and non-diapausing *Doryctobracon areolatus* (Hymenoptera, Braconidae), a parasitoid of *Anastrepha* spp. (Diptera, Tephritidae) fruit flies

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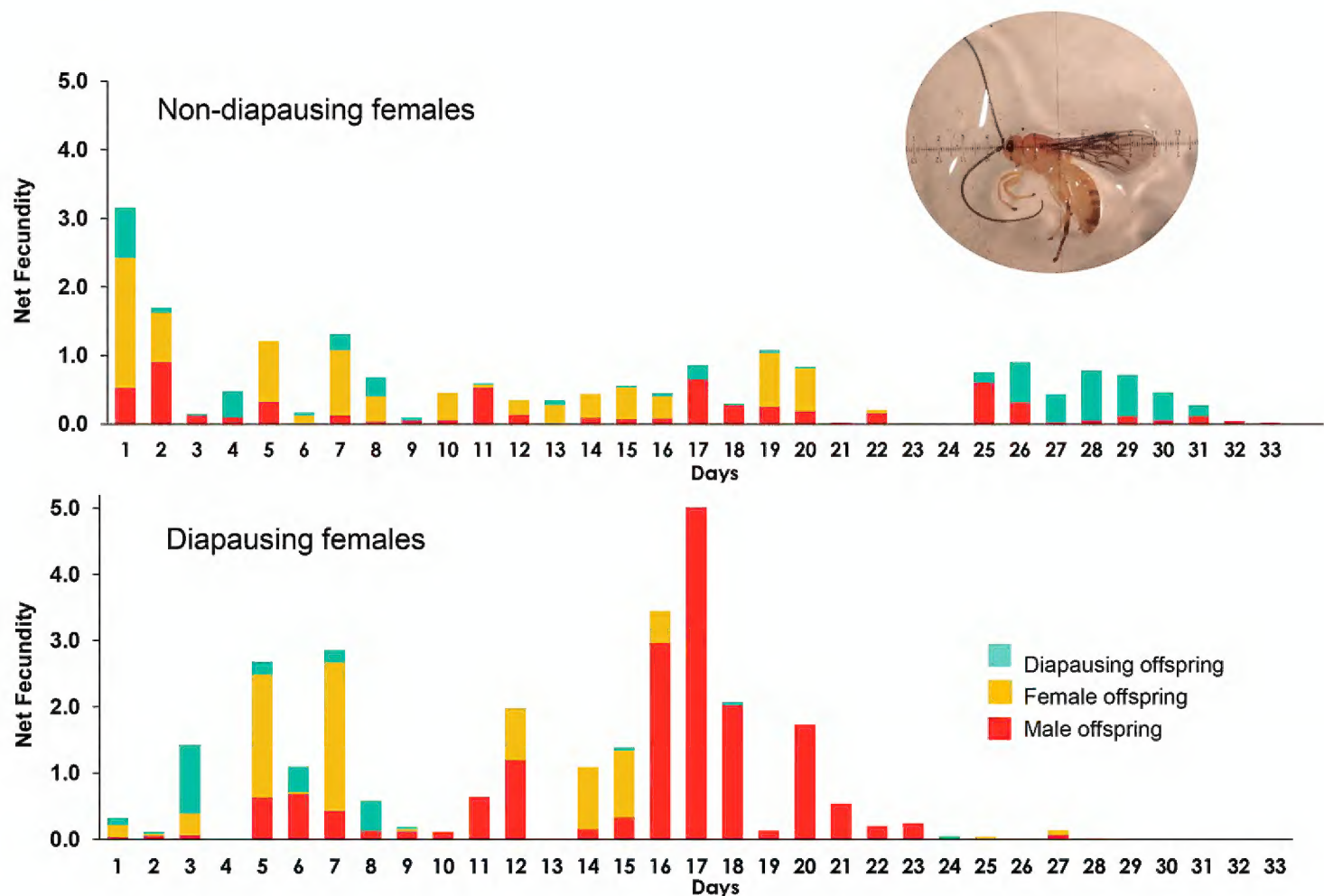
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Abstract

Doryctobracon areolatus (Szépligeti), a solitary endoparasitoid native to the Neotropics, attacks eggs and early instar larvae of *Anastrepha* fruit flies, and can enter diapause under tropical and subtropical conditions. We aimed to test if biological attributes, such as size, flight ability, starvation resistance, longevity and fecundity of diapausing individuals differ from those of non-diapausing ones. Parasitoids were obtained from a laboratory colony reared on *Anastrepha ludens* (Loew) larvae. Parasitized host puparia were sorted in two cohorts according to their diapause condition. Developmental time from egg to adult ranged from 18 to 31 days in non-diapausing parasitoids, and 70 to 278 days for diapausing individuals. Pupal weight and adult measurements were higher in non-diapausing than in diapausing parasitoids. There were no differences in adult longevity, starvation resistance, and emergence between diapausing and non-diapausing wasps. Flight ability and fecundity rates were greater in the non-diapausing than in the diapause cohort. The proportion of female offspring was greater in the non-diapausing cohort (42.5%), whereas in the diapausing cohort the male offspring proportion was greater (62.4%). Both cohorts produced diapause offspring, but the non-diapausing cohort produced more (26.6%) than the diapausing one (9.1%). Maternal age had a significant effect on the proportion of diapause offspring: in 26 to 34 days old non-diapausing females, 78.9% of their offspring entered into diapause. These results confirmed that diapause affects the

biological attributes of *D. areolatus*. The observed differences contribute to better understand the diapause influence on the colonization and rearing process of this species and its use as biocontrol agent.

Graphical abstract



Keywords

Braconidae, fecundity, flight ability, size, Summer diapause, survival, Tephritidae

Introduction

Tephritid fruit fly parasitoids are grouped in five families of Hymenoptera: Braconidae, Diapriidae, Eulophidae, Figitidae, and Ichneumonidae (Ovruski et al. 2000). This represents a wide group that exploits different immature stages of their hosts (eggs, larvae, and pupae) with important variations in their biology and behaviour (Godfray 1994). Since these parasitoids are holometabolous, each species has a different rate of development, which also largely depends on the host (stage, instar, condition). Some species may diapause (Aluja et al. 1998; Carvalho 2005; Ovruski et al. 2016).

Diapause is defined as a type of dormancy in which metabolic and developmental arrest occur in the life cycles of many invertebrates (Mansingh 1971). This allows them to survive in periods of environmental adversity, exploit fluctuating seasonal resources, and synchronize their reproduction (Košťál and Denlinger 2011). Diapause is considered an adaptive strategy in insects that live in temperate regions for winter survival

(Denlinger 2002; Košťál and Denlinger 2011). However, it has also been observed in organisms that live in tropical regions (Denlinger 2002). Several species of tephritid fruit fly parasitoids native to the Neotropics have been reported to diapause: *Doryctobracon areolatus* (Szépligeti), *Doryctobracon brasiliensis* (Szépligeti), *Utetes anastrephae* (Viereck), *Opius bellus* (Gahan) as well the introduced species *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), *Aganaspis pelleranoi* (Brèthes) and *Odontosema anastrephae* (Borgmeier) (Hymenoptera: Figitidae) (Aluja et al. 1998; Carvalho 2005; Ovruski et al. 2016). It has been reported that *D. areolatus* is the species that diapauses with greater frequency and with the longest duration: up to 11 months in fruits of *Spondias mombin* L. (Anacardiaceae) in Mexico (Aluja et al. 1998), and 414 days in fruits of *Eugenia uniflora* L. (Myrtaceae) in Brazil (Carvalho 2005).

The genus *Doryctobracon* Enderlein, 1920, is endemic to the Americas (Ovruski 2003). *Doryctobracon areolatus* is a solitary endoparasitic koinobiont wasp that is widely distributed from Mexico to Argentina and is present in Florida (Wharton and Marsh 1978; Murillo et al. 2015). This species can oviposit in eggs and larvae of 1st, 2nd, and 3rd instars of *A. obliqua* (Macquart) (Murillo et al. 2015). Besides, it has been reported parasitizing *A. ludens* Loew, *A. striata* Schiner, *A. fraterculus* Wiedemann, *A. serpentina* Wiedemann, *A. bahiensis* Costa Lima, *A. crebra* Stone, *A. spatulata* Stone and *Rhagoletis pomonella* Walsh, in Mexico (Aluja et al. 2013).

Doryctobracon areolatus developmental time, when it does not diapause, is 27 days (Murillo et al. 2015); adults usually emerge in synchrony with their host. Under laboratory conditions and optimal diet, adult females live around 20 days (Stuhl et al. 2011; Aluja et al. 2013). This neotropical species shares an evolutionary history with *Anastrepha* fruit flies (Aluja et al. 1998; Ovruski et al. 2000; Carvalho 2005).

The study of diapause in fruit fly parasitoids is relevant not only because of its potential to infer evolutionary relationships, but also to provide insights into the use of these species as biocontrol agents. Biological characteristics of diapausing individuals such as reproductive capacity, tolerance to environmental stress or flight ability can influence their use in biological control projects (Denlinger 2002, 2008; Košťál and Denlinger 2011).

Our aim in this research was to determine if there are differences in *Doryctobracon areolatus* between biological attributes (size, flying ability, starvation resistance, longevity, fecundity, offspring sex ratio and diapause frequency) of individuals which diapause and directly developing individuals, to infer if this condition influences the fitness of this species, and the implications for its mass rearing.

Methods

Biological material

Parasitized host puparia and adult parasitoids came from the *D. areolatus* colony that is maintained in the Biological Control laboratory of the Moscafrut Program (SADER-SENASICA), located in Metapa, Chiapas, Mexico, which at the time of bioassays had

23 generations under laboratory rearing conditions. Second instar larvae of *A. ludens* were used as hosts. Puparia were placed in 30 ml plastic containers, covered with organza fabric to allow ventilation. They were kept on a coconut fiber substrate that was kept slightly humid with water applied by spraying until adult emergence. Laboratory conditions were 24 ± 1 °C temperature, 80–90% relative humidity and a 12:12 L:D cycle.

Developmental time

Two cohorts were obtained based on the type of development of the parasitoids: host puparia with parasitoids without diapause (direct development), and host puparia with evidence of parasitoids in diapause (hereafter non-diapausing and diapausing, respectively). The puparia containing larvae of diapausing parasitoids were distinguished by observing the 3rd instar parasitoid larva inside the host pupa under a stereomicroscope. We recorded the developmental time for both cohorts.

Pupal and adult measurements

Fly puparia were individually weighed using an analytical scale (Ohaus, Pine Brook, NJ) and then placed in plastic containers with 24 independent cells. Each cell was conditioned with lightly moistened coconut fiber substrate, where they remained under laboratory conditions until adult emergence.

Pupal and adult measurements were made with a stereomicroscope (Carl Zeiss, Stemi 2000C) fitted with a scale in the right eyepiece. Thirty host puparia containing parasitoid larvae of each physiological condition were randomly selected and the width and length, from the end of the buccal carinae to the end of the anal pore, were measured. For adult measurements, the cells were checked daily, recording for each emerged parasitoid the date and sex. Each individual was placed in a 1.5 ml vial with an 80% alcohol solution. We measured: 1) length of the left posterior tibia, 2) length of the left wing, 3) mesosoma length, 4) metasoma length, 5) antenna length, and 6) ovipositor length (Sagarra et al. 2001).

Emergence and flight ability

Samples of 100 diapausing and 100 non-diapausing pupae were placed inside a 10 cm diameter X 10 cm height PVC tube, painted black, with the inner wall of the tube covered with neutral talcum powder to prevent the outflow of non-flying parasitoids (as in FAO/IAEA/USDA 2019). These devices were placed inside a 65 × 65 × 45 cm metal frame cage covered with organza fabric (SENASICA 2012). From the beginning of adult emergence, observations were made every 12 hours. Adult parasitoids that were able to fly out of the tube were collected and their number and sex were recorded. The number of non-emerged parasitoids, parasitoids that opened

the puparium but could not get out of it, and non-flying parasitoids (those that despite having emerged completely from the puparium did not manage to leave the tube) were recorded (SENASICA 2012). The number of parasitoids that remained in diapause was also recorded. The number of diapausing parasitoids was subtracted from the initial number to determine the percent of emergence and percent of flyers. There were two replicates for direct development individuals and three replicates for diapausing individuals.

Longevity and starvation resistance

Adults that emerged from both diapausing and non-diapausing larvae were individually placed in 10×12×16 cm plastic cages. Honey embedded in towel paper placed on a plastic lid (1.5 × 0.07 cm) was provided as food (Montoya et al. 2012). Water was supplied in 20 ml plastic containers with a cone of absorbent paper. Thirty males and 30 females of each condition were observed. Daily, the number of dead individuals, type of development and their sex were recorded.

For starvation resistance, at emergence, 30 males and 30 females for each type of development (direct development and diapause) were placed in plastic cages (10×12×16cm) without food and water. Daily, the cages were checked and the dead parasitoids were collected and recorded, noting their type of development, sex and age.

Fecundity

Forty pairs of adults emerged from both diapausing and non-diapausing larvae were used. Each pair was placed in a 10×12×16 cm plastic cage. The individuals were provided with food (honey) and water as described above. The food was changed twice a week. To evaluate fecundity we used artificial oviposition units made of a guava fruit (*Psidium guajava* L.) (Myrtaceae) (Aluja et al. 2009, López-Arriaga et al. 2020), where the mesocarp and seeds were removed and a layer of approximately 5 mm of epicarp was left. The space inside the fruit was filled with a 2.5 cm in diameter plastic sphere and 30 *A. ludens* larvae of second instar mixed with larval food. The spheres had a hole in the center through which a piece of galvanized wire 7 cm long was passed, to hang the device inside the cage. One oviposition device was placed in each cage and they were replaced every 24 hours until the death of the female.

Anastrepha ludens larvae were removed from the oviposition devices and placed in containers with larval diet for seven more days. Then, the mature larvae were separated from the food with a sieve and water and placed in plastic bottles with moist coconut fiber to promote pupation. At emergence, the number and sex of the emerged adult parasitoids were recorded. In the case of non-emerged pupae, they were examined under a stereomicroscope to determine if they were in diapause, dead or were unparasitized *A. ludens* pupae. For each female we recorded the number of offspring produced per day, noting males, females and diapausing individuals.

Statistical analysis

Differences in developmental time (mean $\pm$ SE) were analyzed by a t test. Morphometric data (mean $\pm$ SE) were analyzed using a canonical multivariate analysis of variance (MANOVA) (Fay and Shaw 2010), and multiple comparisons were made using the first two canonical variables (Friendly and Fox 2017). Flight ability (mean $\pm$ SE) was analyzed by a t test. Longevity data were subjected to a survival analysis (Log-rank) (Therneau and Grambsch 2000) with interval-censored data and multiple comparisons by orthogonal contrasts with Bonferroni correction, with a level of significance of 5%. Life tables were constructed with data from the survival and fecundity tests. The survival of females was analyzed by an asymptotic Log-rank test for interval-censored data (Therneau 2015). All analyses were performed using the Software R version 3.4.2. (Venables and Ripley 2002; Fox 2005, 2017; Fox and Bouchet-Valat 2017; R Core Team 2017).

Results

Developmental time

A total of 5,832 host puparia with evidence of diapausing parasitoids, and 934 puparia with non diapausing parasitoids were used in the bioassays. Developmental time from egg to adult, which was from the exposure of the host (*A. ludens* second instar larvae) to adult emergence, ranged from 70 to 278 days for diapausing parasitoids and from 18 to 31 days in non-diapausing ones (Fig. 1). Table 1 shows the mean ($\pm$ SE) parasitoid development time and mean weight ($\pm$ SE) of parasitized host puparia that yielded female and male parasitoids in each development condition.

Pupal and adult measurements

The multivariate canonical analysis, considering the length, width and weight of the host puparia containing wasps in diapause and wasps without diapause, indicated a significant interaction of sex and type of development (Manova, $F_{3,115} = 3.86$, $P < 0.01$). The host puparium weight of non-diapausing parasitoids was greater than that of diapausing ones. However, host puparia of diapausing female parasitoids tended to be longer and wider, but the only significant difference was in puparium length when compared with non-diapausing females. Likewise, host puparia from which female parasitoids emerged were heavier, longer and wider than the puparia containing males (Table 1, Fig. 2).

Regarding parasitoid size, statistical differences were found in the type of development ($F_{5,74} = 7.06$, $P < 0.0001$) and sex ($F_{5,74} = 17.78$, $P < 0.0001$), but there was not a significant interaction between these two factors ($F_{5,74} = 1.07$, $P > 0.05$). Parasitoids directly developing had longer tibia, wing, abdomen, and antenna, compared to parasitoids that diapaused (Table 1, Fig. 3A). Among females, non-diapausing individuals

Table 1. Developmental time and morphological measurements of non-diapausing and diapausing *Doryctobracon areolatus* parasitoids and host puparia. SE: Standard error, n = sample size. Values followed by different letters in each row are significantly different ($P < 0.05$, canonical discriminant analysis).

Type of development	Non-diapausing				Diapause			
	♂		♀		♂		♀	
Sex	Mean $\pm$ SE	n	Mean $\pm$ SE	n	Mean $\pm$ SE	n	Mean $\pm$ SE	n
Parameter								
Development time (days)	21.86 $\pm$ 0.16 c	134	23.35 $\pm$ 0.15 b	179	191.60 $\pm$ 1.62 a	384	188.85 $\pm$ 1.76 a	317
Puparia weight (mg)	13.1 $\pm$ 0.5 ab	29	15.0 $\pm$ 0.6 c	37	12.0 $\pm$ 0.4 ab	32	14.0 $\pm$ 0.5 a	26
Puparia length (mm)	6.25 $\pm$ 0.15 ab	29	6.39 $\pm$ 0.09 c	37	6.24 $\pm$ 0.06 ab	32	6.52 $\pm$ 0.08 a	26
Puparia width (mm)	2.84 $\pm$ 0.03 a	29	2.88 $\pm$ 0.04 a	37	2.81 $\pm$ 0.03 a	31	2.92 $\pm$ 0.04 a	24
Adults								
Tibia length (mm)	1.53 $\pm$ 0.02 a	27	1.56 $\pm$ 0.02 a	28	1.46 $\pm$ 0.03 b	13	1.52 $\pm$ 0.02 a	14
Wing length (mm)	4.88 $\pm$ 0.04 b	27	5.13 $\pm$ 0.08 a	28	4.48 $\pm$ 0.07 c	13	4.89 $\pm$ 0.04 b	14
Thorax length (mm)	2.03 $\pm$ 0.03 a	27	2.13 $\pm$ 0.04 a	28	1.99 $\pm$ 0.03 a	13	2.29 $\pm$ 0.24 a	14
Abdomen length (mm)	3.37 $\pm$ 0.07 a	27	3.30 $\pm$ 0.06 a	28	3.31 $\pm$ 0.07 a	13	3.09 $\pm$ 0.09 b	14
Antenna length (mm)	7.64 $\pm$ 0.10 a	27	7.27 $\pm$ 0.10 b	28	7.08 $\pm$ 0.10 b	13	6.64 $\pm$ 0.11 c	14
Ovipositor length (mm)	–		4.92 $\pm$ 0.07 a	28	–		4.69 $\pm$ 0.24 b	14

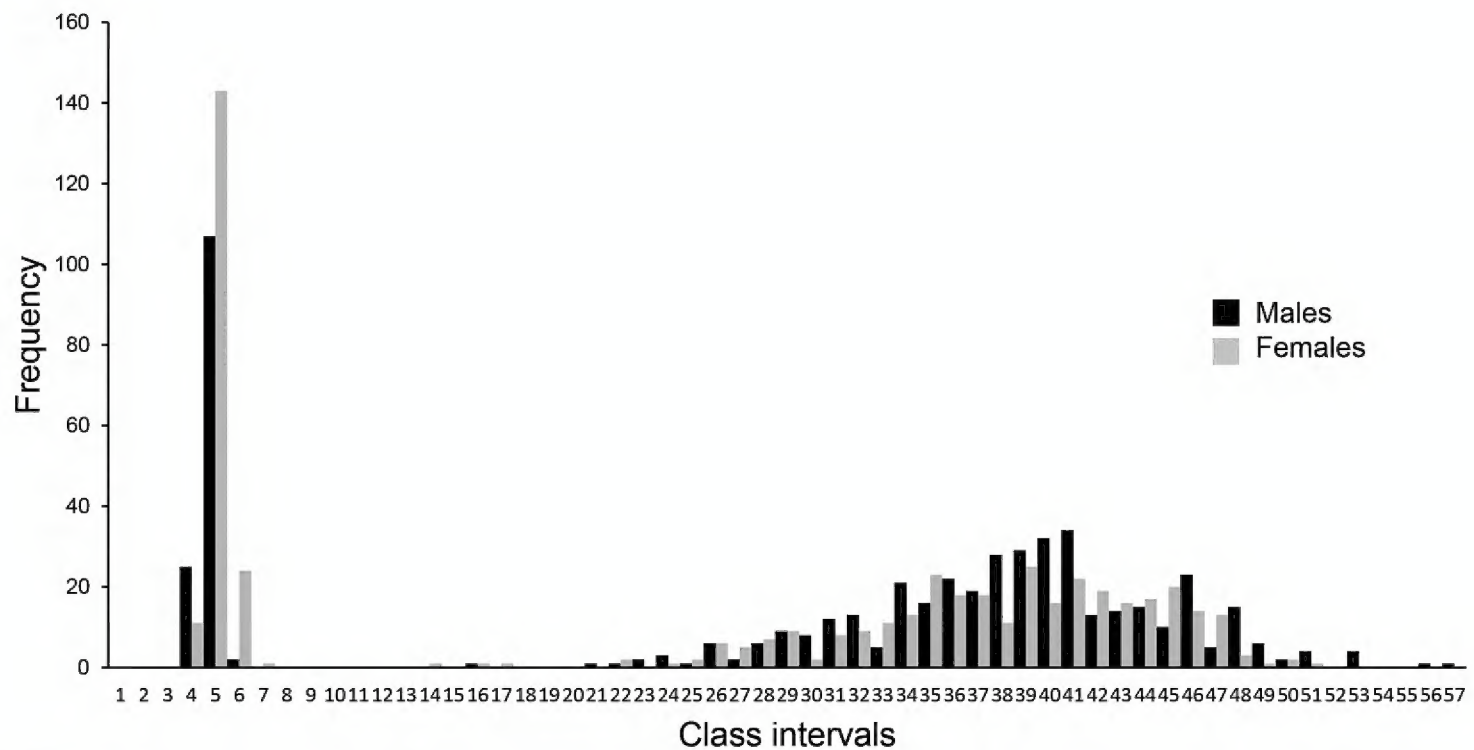


Figure 1. Duration of development of non-diapausing and diapausing *Doryctobracon areolatus* females and males, parasitizing *Anastrepha ludens* larvae.

were larger than diapausing ones in wing, abdomen, antenna, and ovipositor lengths (Table 1, Fig. 3B). Male parasitoids from direct development had longer tibia, wing and antenna than diapausing males. Regardless of the type of development, females were larger than males in tibia, wing, and mesosoma length (Table 1, Fig. 3C). The canonical analysis, considering all six adult parasitoid measurements, showed that non-diapausing parasitoids were significantly larger than diapausing ones.

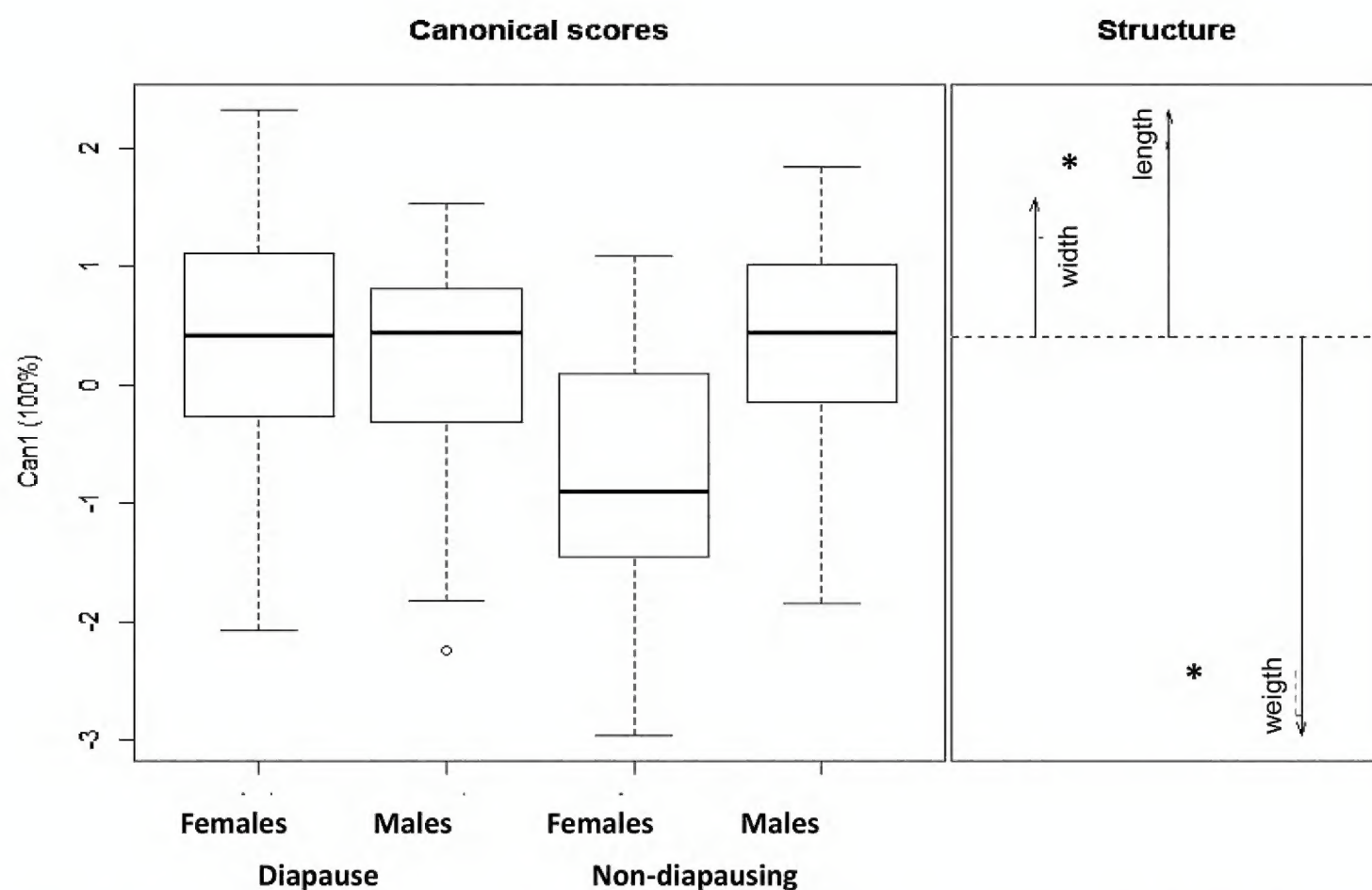


Figure 2. Canonical analysis of morphological data from puparia containing non-diapausing and diapausing male and female *Doryctobracon areolatus* parasitoids. The asterisk (*) indicates a significant difference.

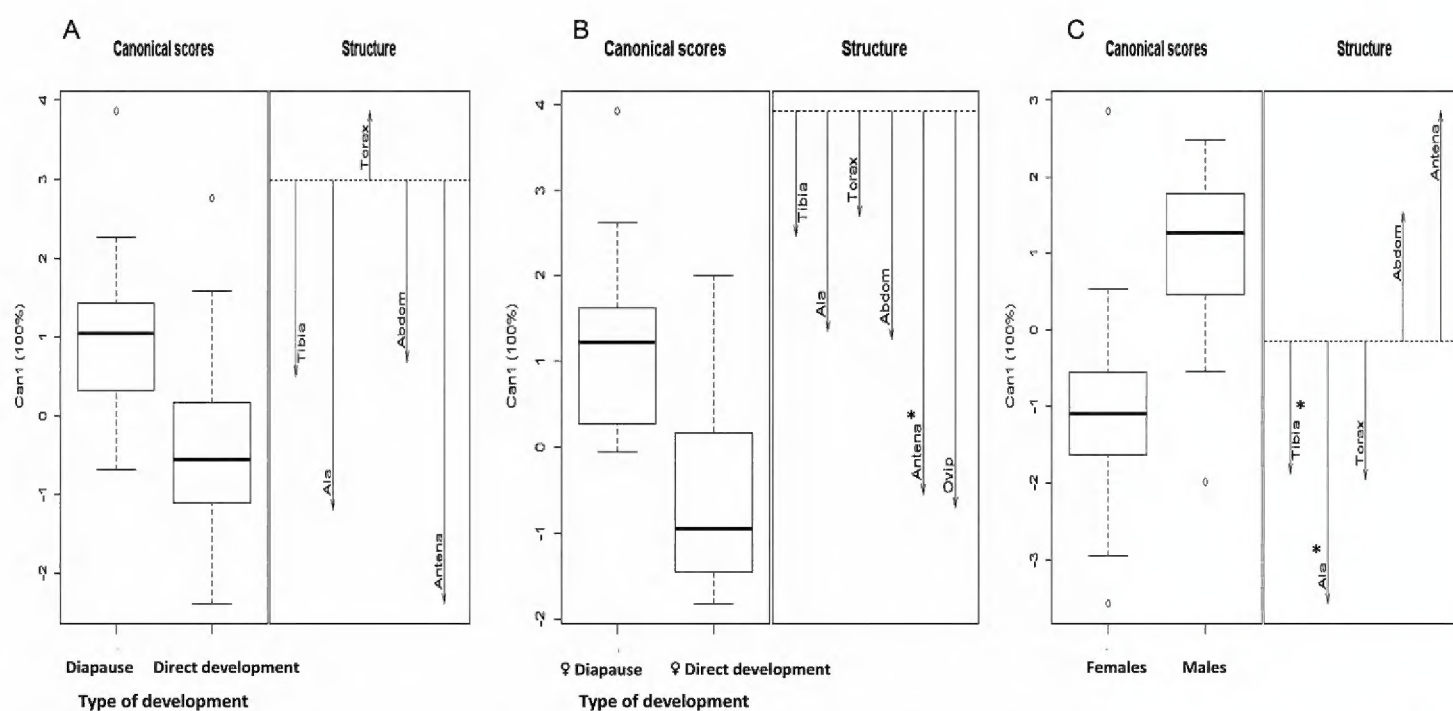


Figure 3. Canonical analysis of adult parasitoids' morphological data from non-diapausing and diapausing *Doryctobracon areolatus*. **A** Comparison by type of development **B** comparison among females **C** comparison between sexes. The asterisk (*) indicates a significant difference.

Emergence and flight ability

Adult emergence rate was higher in non-diapausing parasitoids ($75.69\% \pm 2.96$) than in diapausing parasitoids ($39.86\% \pm 9.69$). However, the difference was not statistically significant (Student t test, $t = 2.82$ df = 3, $P > 0.05$). The percentage of flying

Table 2. Flight ability and emergence rate of non-diapausing and diapausing *Doryctobracon areolatus* parasitoids. Emergence rate is the proportion of adults emerged from 100 host puparia. Percentage of flyers is the proportion of parasitoids capable of flying from 100 host puparia. SE = Standard Error. Values followed by different letters in each column are significantly different ($t = 4.03$, $df = 3$, $P < 0.05$).

Cohort	Replicates	Emergence rate (%) $\pm$ SE	Fliers (%) $\pm$ SE
Non-Diapausing	2	75.69 $\pm$ 2.96 a	55.56 $\pm$ 3.54 a
Diapausing	3	39.86 $\pm$ 9.69 a	23.36 $\pm$ 5.36 b

parasitoids from direct development (55.56% $\pm$ 3.54) was significantly higher than in diapausing parasitoids (23.36% $\pm$ 5.36) ($t = 4.03$, $df = 3$, $P < 0.05$) (Table 2).

Longevity and starvation resistance

There were significant differences in survival between starved individuals and those provided with food (Log-rank test, $\chi^2 = 98.46$, $df = 7$, $P < 0.001$). When food was provided, diapausing females showed the greatest mean longevity (24 days, Table 3). Longevity of diapausing females was significantly different from the longevity of diapausing males ($Z = 3.46$, $P = 0.0005$) but it was not different from that of non-diapausing males and females.

No significant differences were found in starvation resistance between diapausing and non-diapausing cohorts, nor between females and males (Table 3).

In the fecundity bioassays when females were provided with hosts, there was no difference in the survival of the females of both conditions ($Z = 1.24$, $P > 0.05$, Fig. 4).

Fecundity

Differences in the reproduction of parasitoids emerged from direct development and diapause were observed, both in the fecundity rates and in the allocation of the offspring. Fecundity was higher for the non-diapausing cohort than for the diapausing one. Non-diapausing females also produced more daughters than diapausing ones and more individuals entering into diapause (Table 4). Females of both cohorts started reproduction from the first day of adult life. In non-diapausing females, daily net fecundity was at its maximum on day 1 (Fig. 5), and their female offspring represented 42.58% of the total offspring. In diapausing females, the peak fecundity was observed on day 17 and males represented 62.54% of the total offspring. Both cohorts produced offspring that entered into diapause, 26.55% in non-diapausing females and 9.12% in diapausing females (Table 4).

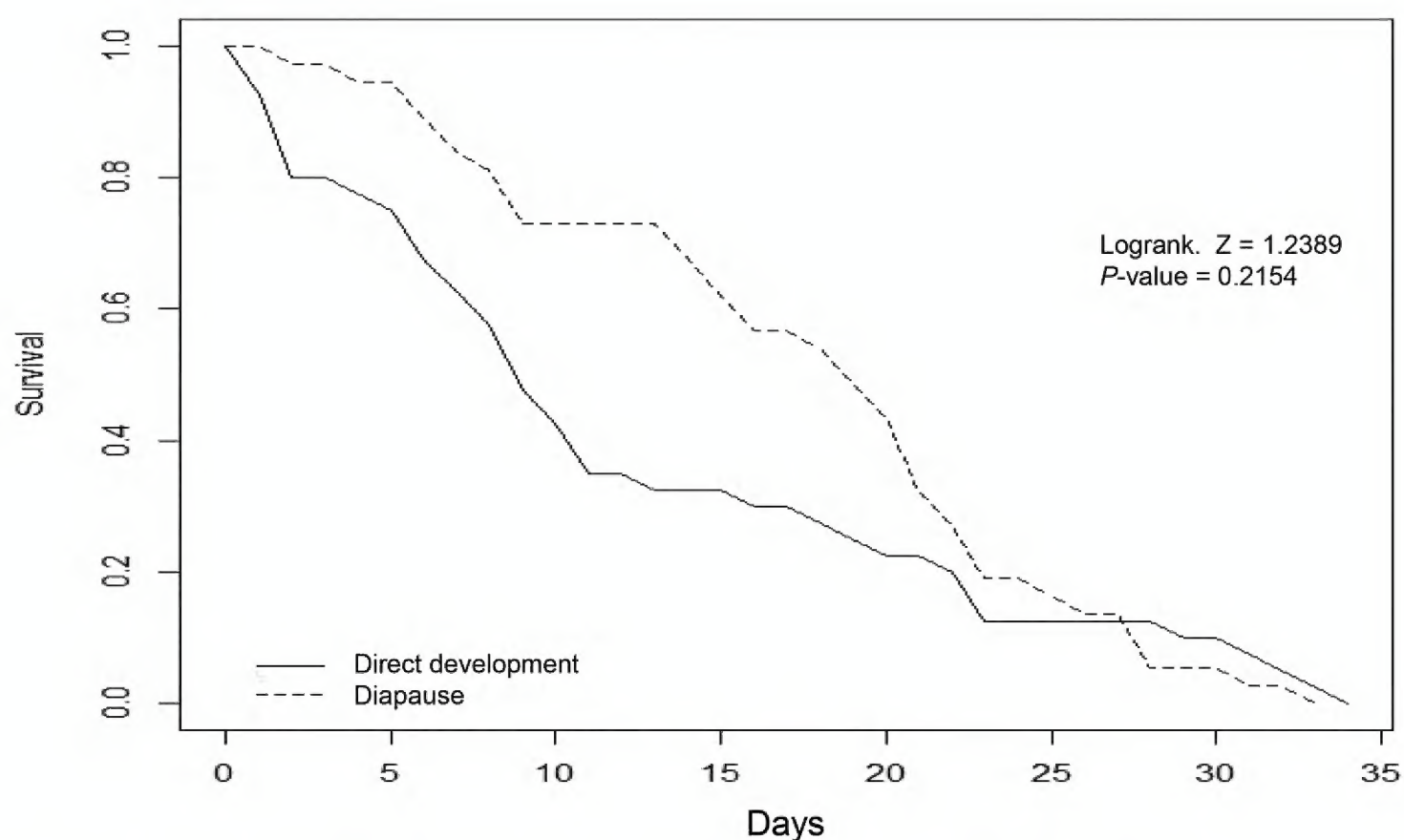
About 67% of both diapausing and non-diapausing pairs produced offspring. Of those pairs with offspring, 86.2% of the non-diapausing cohort and 100% of the diapausing cohort produced males, whereas 62% and 48%, respectively, produced females. There were no differences in the number of non-diapausing and diapausing pairs that produced daughters ($\chi^2 = 1.07$, $P > 0.05$). The fraction of

Table 3. Mean longevity ($\pm$ SE) and starvation resistance (in days) in non-diapausing and diapausing *Doryctobracon areolatus* parasitoids.

Sex	Type of development	Longevity (days)			
		With food	n	Without food	n
♀	Non-diapausing	14.77 $\pm$ 2.27 ab	43	4.18 $\pm$ 0.33 a	55
	Diapausing	24.00 $\pm$ 3.04 a	30	5.40 $\pm$ 0.27 a	45
♂	Non-diapausing	12.09 $\pm$ 1.48 ab	47	4.40 $\pm$ 0.40 a	43
	Diapausing	10.67 $\pm$ 1.35 b	52	4.33 $\pm$ 0.24 a	52

Table 4. Fecundity rates (female offspring per female) of non-diapausing and diapausing *Doryctobracon areolatus* parasitoids and fraction of females, males and diapausing offspring.

	Non-diapausing	Diapausing
Gross fecundity (daughters / female)	19.99	10.92
Net fecundity (daughters / female)	8.47	8.00
Male offspring (%)	30.87	62.54
Female offspring (%)	42.58	28.49
Diapausing offspring (%)	26.55	9.12

**Figure 4.** Female survival of *Doryctobracon areolatus* parasitoids in the fecundity bioassays.

pairs that produced offspring that entered into diapause was 58% and 40% for the non-diapausing, and diapausing cohorts, respectively. Maternal age in the non-diapausing cohort had an important effect on the production of offspring that entered into diapause. Over 78% of the offspring of 26 to 34 days-old females from

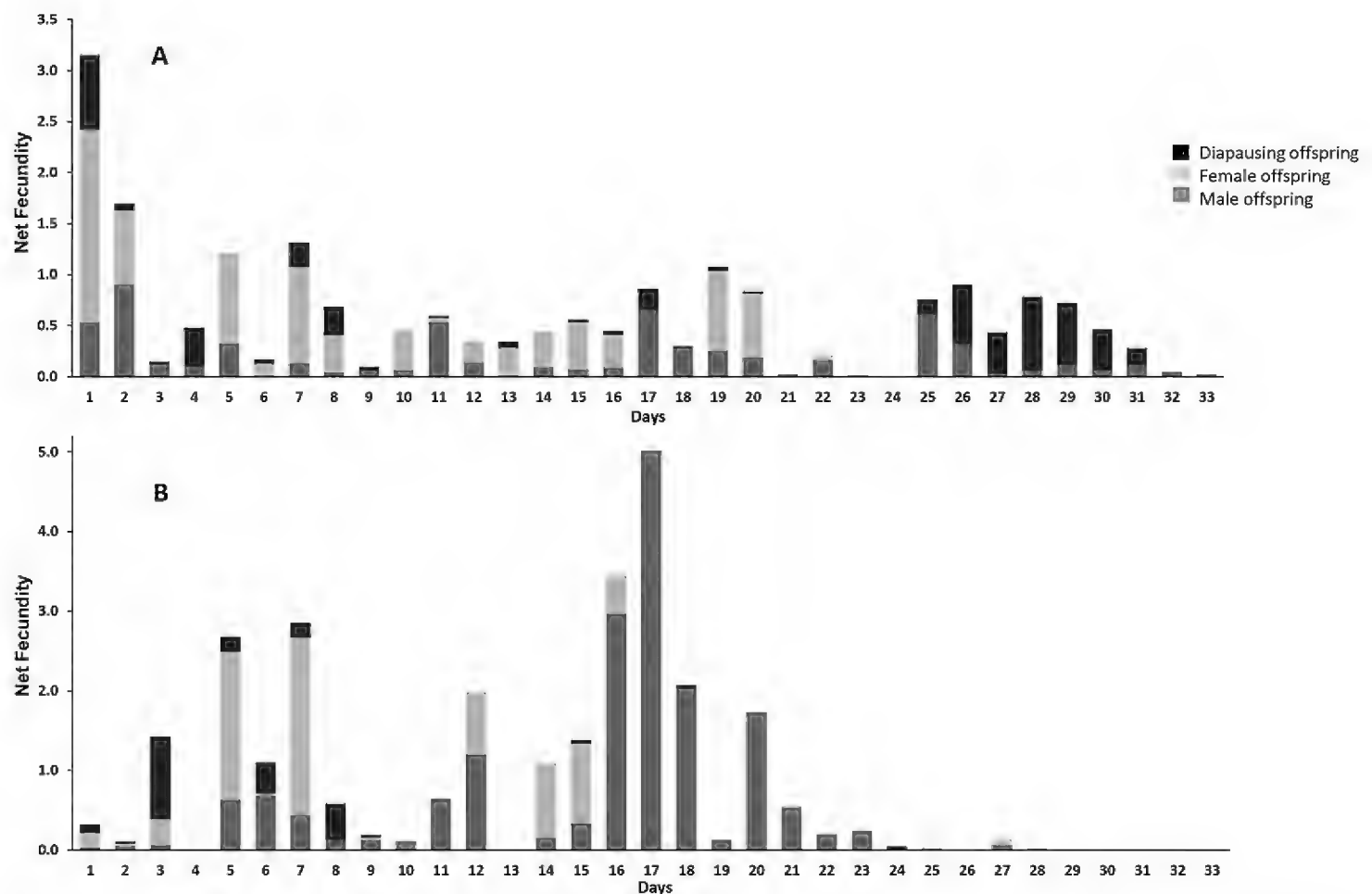


Figure 5. Net fecundity of *Doryctobracon areolatus* females from **A** non-diapausing and **B** diapausing cohorts.

the direct development condition entered into diapause, and the rest were only males (Fig. 5). Diapausing females that reached this age range did not produce offspring entering diapause.

Discussion

Diapausing and non-diapausing *D. areolatus* individuals differed in pupal and adult size, flight ability, fecundity, and reproductive dynamics, whereas there were no differences in their percent of adult emergence, starvation resistance and adult survival when provided with food. Interestingly, females from both cohorts produced diapausing offspring. However, non-diapausing females produced a higher percentage of diapausing offspring than diapausing females, particularly at old ages (26 to 34 days-old). The physiological, behavioral and evolutionary reasons for this are new research questions.

Under our laboratory-controlled conditions and using mass-reared *A. ludens* as hosts, we found that diapause in *D. areolatus* has been maintained after 23 generations, suggesting a genetic component. The developmental time of diapausing individuals ranged from 70 to 281 days, which is shorter than those reported for this species when they were collected in the field in Mexico, Brazil and Argentina (Aluja et al. 1998; Carvalho 2005; Ovruski et al. 2016). These reports included wild individuals coming from different fruit fly species hosts (*A. obliqua*, *A. ludens*, *A. fraterculus*, *A. serpentina*

and *A. striata*), infesting different fruit species (*E. uniflora*, *S. mombin*, *P. guajava* L. and *Tapirira mexicana* March. (Anacardiaceae)), under varied climatic conditions.

Another factor affecting diapause duration is the metabolic reserves of individuals (Ishihara and Shimada 1995; Hahn and Denlinger 2007), because only those larvae with enough nutritional resources will survive this period (Ellers and van Alphen 2002). Despite we found that diapausing individuals had a lower pupal weight, – which could mean lower fat reserves – and adults emerging from diapause were smaller, there were no difference in adult percent emergence between the two cohorts. Aluja et al. (1998) and Ovruski et al. (2016) previously noted the smaller larval and pupal weight of diapausing individuals, but no comparisons had been made on adult dimensions. Wings also were longer in non-diapausing wasps and this could be associated with the greater flight ability observed in this cohort. Kölliker-Ott et al. (2003) proposed that wing size, shape and asymmetry influenced the field fitness in *Trichogramma* egg parasitoids. The changes in body size and fat reserves are among the most notorious characteristics of diapausing individuals, which is related to the energetic demands and fitness costs associated with diapause (Ellers and van Alphen 2002; Hahn and Denlinger 2007).

It is generally agreed that diapause represents a strategy to cope with adverse environmental conditions, including a shortage of hosts. In this context, and contrary to the expectation of an increased starvation resistance in diapausing individuals, our results showed that diapausing and non-diapausing parasitoids had similar survival rates when deprived of food. However, females emerging from diapause and provided with food and water lived longer than non-diapausing females (Table 3, Fig. 4), although difference was not statistically significant. This suggests that adults emerging from diapause could be more efficient using the nutritional reserves.

The higher proportion of female offspring produced by non-diapausing females could be attributed to a lower mating rate in the diapause cohort, thus increasing the production of parthenogenetic male individuals. The higher fecundity of the non-diapausing cohort and the sex ratio biased to females indicate that these parasitoids will show greater population growth rates than diapausing ones. This decrease in reproduction may represent a trade-off for diapause affecting the adult fitness components (Ellers and van Alphen 2002; Hahn and Denlinger 2007; Sadakiyo and Ishihara 2012).

Our most important finding was the effect of mother age of non-diapausing females on the production of offspring entering into diapause. Females of 26 to 34 days old, produced 78% of the offspring that entered into diapause. This suggests that diapause in *D. areolatus* has a hard-wired genetic component and may represent an adaptation to host scarcity in the field (Denlinger 1986). Although the frequency of diapause in *D. areolatus* has been previously associated with the fruit species used by the fruit fly host (Ovruski et al. 2016), in this study, we used the same fruit species (*P. guajava*) and we still found differences between diapausing and non-diapausing individuals respect to maternal age and the proportion of offspring entering into diapause. Some studies proposed that parents might determine whether their offspring

enters into diapause (Denlinger 2002; Hahn and Denlinger 2007). Environmental factors affecting the parental generation may determine the production of diapausing offspring (Saunders 1965). In this sense Gonçalves et al. (2018) showed that temperature influence the immature development and demographic parameters of a Brazilian population of *D. areolatus* parasitizing *A. fraterculus*, but it remains to test whether temperature or other environmental factors affect the frequency of diapause in this species. Our *D. areolatus* population has been exposed to *Anastrepha ludens* larvae in guava fruits under laboratory conditions for 23 generations. During this time, diapausing individuals were systematically sorted out using only non-diapausing individuals to maintain the laboratory colony. Despite the constant environmental conditions and this selection against diapause, the proportion of diapausing individuals did not vary significantly through generations. According to Rahimi-Kaldehy et al. (2018) maternal age together with other abiotic factors (i.e. temperature, humidity, light) can affect the percentage of offspring entering into diapause in *Trichogramma brassicae* Bezdenko, where the percentage of diapause decreases as maternal age increases. An inverse effect was reported for several Pteromalidae parasitoids, such as *Spalangia* sp. and *Nasonia vitripennis* (Walker), where the probability of larvae entering diapause increases if they were born from eggs laid by aging females (Simmonds 1948; Saunders 1965). The same occurred in our study with *D. areolatus*, where the oldest females, emerged from non-diapausing pupae, produced mainly offspring that entered into diapause.

We concluded that diapause in *D. areolatus* has effects on some biological attributes that can be trade-offs in the parasitoids fitness, such as pupal and adult size, flight ability and reproduction. We also found that maternal age has an important effect on the proportion of offspring entering diapause. This represents baseline knowledge to understand how diapause affects the population dynamics of this species and the possible implications in the implementation of the mass rearing and biocontrol applications. The presence of diapausing individuals as part of the offspring of the released population could improve the effectiveness of biocontrol strategies during unfavorable environmental conditions emerging at the optimal time.

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